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Report Prepared By: Dr. M. Michael Sigel Date: February 8, 1955

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COMPRACTOR: The Children's Hospital of Philadelphia

Dr. M. Michael Sigel - Laboratory and Field Studies NVESTIGATORS:

> Dr. Louis Grant, University College of West Indies Mona, Jamaica - Laboratory Studies

Prof. G. H. C. Ovens, University College of West Indies Mona, Jamaica - Chronic Infections

Dr. R. Miles, University College of West Indies Mona, Jamaica - Chronic Infections

Dr. E. Sutherland, V. D. Clinics, Jamaica, - Acute Infections

Dr. Carroll F. Burgoon, Children's Hospital of Philadelphia, Philadelphia, Pennsylvania - Field Studies

Dr. Jose Vanella - Laboratory Studies

"Studies on the Psittacosis-Lymphogranuloma Veneraum TITLE OF PROJECT: Group"

Objectives: 1. To search for a specific complement fixing antigen. (Investigations completed March 1953)

> 2. To study mode of growth and multiplication and other biologic characteristics of this group of agents. (Major part of investigations completed prior to July 1953)

3. To study the effect of therapeutic agents (antibiotics) on these viruses and on the diseases caused by them.

Abstract of Results:

A. Work Completed and Previously Reported:

This work has been reported through the medium of semi-annual, quarterly and special reports, and publications. The following is a list of publications from 1949 to 1954:

Differences in the hemagglutinating and antigenic properties of strains of influenza virus isolated from one outbreak. M. M. Sigel. J. Immunol., 62, 81, 1949.

The virus diagnostic research laboratory. Considerations of its importance in maintaining community health based on two years actual operation. M. M. Sigel, W. Henle and T. F. McNair Scott, Penna. Med. J., 52, 372, 1949.

Q fever: Report of a case in Pennsylvania. 0. H. Janten, A Bondi and M. M. Sigel. Annals of Int. Med., 30, 180, 1949.

Immunologic response of hamsters to influenza virus strains. M. M. Sigel, E. G. Allen, D. J. Williams and A. J. Girardi. Proc. Soc. Exp. Biol. and Med., 72, 507, 1949.

The recurrence of influenza A prime in a boarding school after two years. M. M. Sigel, A. W. Kitts, A. B. Light and W. Henle, J. of Immunol., 64, 33, 1950.

Q fever in a wool and hair processing plant. M. M. Sigel, T. F. McNair Scott, W. Henle and O. H. Janten. Am. J. Pub. Health, 40, 524, 1950.

The role of the virologist in the diagnosis of acute infectious diseases. M. M. Sigel, Med. Clinics of North America. 34, 1605, 1950.

Aureomycin therapy in lymphogranuloma venereum. A. Fletcher, M. M. Sigel and H. A. Zintel. Archives of Surgery, 62, 239, 1951.

Studies on the psittacosis-lymphogranuloma group. I. The pattern of multiplication of meningopneumonitis virus in the allantois of the chick embryo. M. M. Sigel, A. J. Girardi and E. G. Allen. J. Exp. Med., 91, 401, 1951.

Preservation of viruses of the psittacosis-lymphogranuloma venereum group and herpes simplex under various conditions of storage.

E. G. Allen, B. Kaneda, A. J. Girardi, T. F. McNair Scott and M. M. Sigel. J. Bact., 63, 369, 1952.

Factors influencing the neutralization of lymphocytic choriomeningitis virus. R. Pollikoff and M. M. Sigel. Bact. Proceedings, 1952.

Studies on the psittacosis-lymphogranuloma group. II. A non-infectious phase in virus development following adsorption to host tissue. A. J. Girardi, E. G. Allen and M. M. Sigel. J. Exp. Med. 96, 233, 1952.

Coxsackie viruses and human disease. Advances in Medicine and Surgery from the Graduate School of Medicine of University of Pennsylvania. M. M. Sigel, W. B. Saunders Company. P. 372, 1952.

Influence of age on susceptibility to virus infections. M. M. Sigel., Annual Review of Microbiology., 6, 247, 1952.

Studies on the psittacosis-lymphogranuloma group. III. The effect of aureomycin on the propagation of virus in the chick embryo. E. G. Allen, A. J. Girardi, M. M. Sigel, and M. Klein. J. Exp. Med., 97, 783, 1953.

The mounting incidence of psittacosis. M. M. Sigel, L. S. Cole, and O.Hunter, am. J. of Pub. Health. 43, 1418, 1953.

Studies on the psittacosis-lymphogranuloma group. IV. Demonstration of specific CF antibodies following absorption of serum. R. Pollikoff and M. M. Sigel. J. of Immunol., 71, 436, 1953.

The reduction of group reactivity of the complement fixing antigen of meningopneumonitis virus by potassium periodate.

M. M. Sigel and R. Pollikoff. Proc. Soc. Exp. Biol. and Med., 84, 517, 1953.

Distribution and persistence of aureomycin in the chick embryo. E. G. Allen, A. J. Girardi, M. M. Sigel, and M. Klein. Proc. Soc. Exp. Biol. and Med., $\underline{82}$, 542 - 1953.

Diagnostic advances at virus laboratory. M. M. Sigel. Research Reviews. July 1949.

The mode of multiplication of meningopneumonitis virus. M. M. Sigel, A. J. Girardi, and E. G. Allen. (Paper given at VI International Congress of Microbiology, Rome, Italy - September 1953)

Recent advances in the differential diagnosis and treatment of infections with viruses of the psittacosis-lymphogranuloma venereum group. M. M. Sigel (Paper read before the 5th International Congress of Tropical Medicine and Malaria, Istanbul, Turkey, 1953)

Papers dealing with influenza and Q fever relate to a previous ONR contract.

B. Work Completed But Not Yet Reported:

- I. Jamaica, British West Indies
 - (1) The effect of antibiotic therapy on LGV (two-year follow-up)

The appendix to this report gives a breakdown and a scoring procedure for the comparison of the effects of several therapeutic agents. It is fully realized that the results and the scores may be open to question and should, therefore, be qualified. The following factors and circumstances may have played an important role in determining the final analysis. (1) The patients varied as regards their integrity and intelligence. This factor would influence their regularity and adherence to treatment schedules as well as visits to the clinic for follow-ups. (2) Because of the high prevalence of the disease it is quite possible that during the period of two years, several infections with LGV may have taken place in a given patient,

thus accounting for persistence or elevation of antibodies over a period of time. We attempted to control this factor by differentiating between serologic patterns consisting of "titer increase with previous decrease" (this presumably signifying a reinfection) and "titer increase without a previous decrease" (this presumably signifying a continuing old infection). Even this differentiation is subject to error because the chances of showing a decrease in titer prior to an increase are greater when the number of blood specimens per patient is greater. Thus, a cooperative patient who presented himself for many follow-ups is more likely to show evidence of an antibody decline than a patient who made few visits to the clinic. (3) There was considerable variation in the number of patients treated with various drugs. The determining factor in selecting patients for different drugs was the amount of drug available. (4) The scoring method was selected arbitrarily. It is believed, however, that it reflects the significant differences in various groups. The positive scores were assigned to changes in antibody titer consistent with improvement or cure. In all of these positive scores, a good initial clinical response is implicit.

Whenever the clinical response was only <u>fair a -l</u> score was assigned to the patient in addition to the score derived from the serologic results. For example, a patient with a fair initial clinical response and a complete serologic reversal was scored -l and +6.

A questionable initial response had a -2 score.

A poor initial clinical response was designated with a <u>-5</u> score.

When two negative scores were justified - only one, the stronger negative, was given.

The results presented in the appendix may be summarized in the following ways.

Based on the complete period of observation, the average scores are:

Aureomycin	2.7
Triplesulfonamide	2.6
Terramycin	1.6
Chloromycetin	1.5
Ilotycin	-0.1
Sulfathiazole	-1.2
Magnamycin	-2.7

If the scoring is limited to patients with regular attendance, the average scores are:

Aureomycin	17/23	3.9
Terramycin	6/8	3.0
Triplesulfonamide	5/1 0	3.0
Ilotycin	12/19	2.2
Chloromycetin	14/18	2.1
Magnamycin	4/7	-1.8
Sulfathiazole	2/11	2.5

In the "irregular" patients or patients where regularity is unknown, the scores are:

Triplesulfonamide	2.0
Aureomycin	-0.8
Chloromycetin	- 0.5

Sulfathiazole	-2.1
Terramycin	-2.5
Ilotycin	-4.0
Magnamycin	-h.0

Based on analysis of serologic patterns during a period of the first 3-6 months following commencement of treatment, the average scores are: (On the basis of serologic pattern alone)

	OVERALL	REGULAR PATIENTS
Aureomycin	3•3	3.3
Triplesulfonamide	3.2	3.4
Terramycin	2.6	3.6
Chloromycetin	1.6	1.7
Ilotycin	1.6	2.3
Magnamycin	-0.7	1.5
Sulfathiazole	1.6	

A similar trend is shown by the following ratios of sera (taken during the period of the first 3 - 6 months) showing a decrease in antibody titers:

Triplesulfonamide	9/9
Aureomycin	13/17
Terramycin	5/7
Chloromycetin	7/13
Ilotycin	7/17
Magnamycin	1/4
Sulfathiazole	2/5

Two findings emerged as by-products of this investigation.

(1) The higher antibody titers usually reflected more severe or more extensive types of infection. (2) It was rather difficult to reduce or eliminate high titers (i.e., 1:320 and higher).

(2) Epidemiological studies

a. Development of antibodies to LGV in school children

As was stated in the summary of the initial phase of study in Jamaica: "Very little is known about the existence of psittacosis or trachoma in Jamaica. It was felt that continued examination of sera from children may throw some light on the question as to what proportion of LGV antibodies represents venereally acquired infections." The findings would also help answer the question as to whether LGV is a truly venereal disease.

Calabar School, Kingston, Jamaica

Starting number 102. At the end of two years, 56.

50 negative*

5 gave fluctuating reactions in the range of 1:10 and negative

1 appeared to show a slight rise from negative to 1:20

St. Joseph School, Kingston, Jamaica

Starting number 49. At the end of two years 21.

21 negative

St. Aloysius School, Kingston, Jamaica

Starting number 18. At the end of 2 years, 8.

7 negative

1 slight rise (negative to 1:10)

Vere

Starting number 80. At the end of 2 years, 37.

- 30 negative
- 2 fluctuating (negative to 1:20 to 1:10, negative to 1:10 to negative)
- 4 slight rise negative to 1:10

Maypen

Starting number 73. At the end of 2 years, 31.

- 24 negative
- 1 fluctuating (negative to 1:20 to negative)
- 5 slight rise negative to 1:10
- 1 significant rise, negative to 1:640

Watermount

Starting number 62. After 2 years, 35.

- 30 negative
- 1 fluctuating (nonspecific to 1:20 to 1:10)
- 1 slight rise, negative to 1:10
- 2 rise, (1 negative to 1:40, 1 negative to 1:80)
- 1 incomplete (negative to positive, exact titer unknown).

Salt Spring

Starting number 62. After 2 years, 33.

- 31 negative
- 2 decrease 1:20 to negative

These results indicate that the antibodies to IGV occur with very low frequency in children as compared to adults in Jamaica. In only four instances can the results be regarded as indicating acquisition of an infection. The significance of slight rises, low titers (1:10 to 1:20) and fluctuating titers remains undetermined. It is planned

there appeared to have been a slight increase or fluctuation. It is also proposed to obtain information about any clinical findings on the children with relative high increases in antibody titers. These observations are in accord with previously stated impressions (see report of January 1954) that the acquisition of antibodies to the psittacosis-LGV group of viruses in Jamaica comes as a result of sexual activity.

b. St. Louis encephalitis

In the course of the investigations on LGV, serologic tests were carried out for the purpose of determining what other virus infections were prevalent in Jamaica. A few sera showed the presence of antibodies to influenza and mumps viruses, and typhus and the spotted fever-rickettsialpox group of rickettsiae (usually in low titers). What was more impressive was the finding that children in the areas of Maypen and Vere had experienced infections with a member of the St. Louis encephalitis group. Because of this finding, it was considered advisable to search for evidence of infection in potential reservoirs and vectors. Field collections of bird and animal bloods and of mosquitoes were therefore carried out in December 1954. Neutralization tests have revealed antibodies to a virus of the St. Louis encephalitis group in the great majority of sera of mules and donkeys, and in several sera of wild birds collected in the areas of Maypen and Vere. Virus

was, however, not isolated from three pools of mosquitoes. These results suggest that SLE virus or another member of its group which is infectious for man, mules and birds is present in Jamaica.

II. Work in the United States

(1) Interaction of Krebs-2 ascites tumor and meningopneumonitis
virus

Although the more recent investigations on this problem were done as part of the activities of the Communicable Disease Center, USPES, the ground work as well as all of the earlier experiments were accomplished with the support of the ONR. The results of this investigation have provided a new concept of virus-tumor interaction. While other virus-tumor systems studied are associated with virus multiplication in the tumor cells and the resulting destruction of the cells, in this system tumor inhibition appears to be the result of competition between virus and tumor cells for a common substrate. This assumption is based on several types of evidence including the observation that under certain conditions it is possible to reverse the phenomenon and cause inhibition of virus multiplication by growing tumor.

(2) Psittacosis virus in the rabbit skin

Attempts were made to establish an allergic type of sensitivity to psittacosis virus in rabbits. Determinations of sensitivity were complicated, however, by the development of reactivity to normal tissue components present in the sensitizing antigens. It was found that interchange of antigen sources (for example, primary sensitization

with virus grown in chick embryo and challenge with virus grown in mouse brain) could not be accomplished because of the relatively low concentration of virus in tissues other than chick embryo membranes and fluids. These experiments yielded, however, an interesting by-product. It was found that psittacosis virus was capable of causing local lesions in the skin of rabbits. These reactions were suppressed by immune serum.

C. Work Not Completed

The epidemiological investigations embracing adults and children and pertaining to the quustions of development, stability, and magnitude of antibody titers to LGV virus require more extensive observations.

The pathogenesis of LGV in the female and the mechanism of development of rectal lesions are still not well understood. The observations by Dr. Miles suggest that the rectal lesions are not the result of spread by way of the pelvic lymphatics. Dr. Miles and a group of gynecologists are interested in pursuing this problem further. Whether it will be possible to continue or extend the therapeutic experiments will depend on the availability of funds and/or drugs.

Some of the completed phases of the work will be published in the near future. It is also planned to prepare a monograph incorporating all of the pertinent studies in Jamaica. This project may have to swait completion of the clinical, gynecological, and surgical studies.

METHOD OF SCORING

- 6 complete reversal
- 5 greater than 8 fold decrease in titer
- 4 8 fold decrease or persistently low titer (persistent negative titers not scored because of uncertainity that case was LCV)
- 3 4 fold decrease (persistent)
- 2 decrease (greater than 4 fold or complete reversal)followed by rise
- 1 fluctuation in titer
- 0 no change in titer
- -l fair initial clinical response
- -2 questionable initial clinical response
- -3 reappearance of gland after 3 months and with no previous decrease in titer
- -4 late symptoms
- -5 poor initial response and/or rupture of gland after 5th day of treatment
- -6 rise in titer beyond 3 months of initiation of treatment and with no previous decrease

AUREOMYCIN

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*Reappearance of adenitis
**Except when at hospital for 6 days at inception of treatment
***Bilateral lymphoadenopathy
filand reappeared. Placed on sulfathiazole

Whenever several titers are given in one box, they usually represent results on successive serum specimens. Neg. signifies < 1:10.

Dosage in capsules: C - Clinic; H - Home Loc. - Location of bubo

R - Regular; I - Irregular; ? - Questionable G - Good; F - Fair; P - Poor - Regularity: Reg. - Regularity: Clinical Response:

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AUREOMICIN (continued)

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Dosage in capsules: C - Clinic; H - Home

Loc. - Location of bubo

Reg. - Regularity: R - Regular; I - Irregular; ? - Questionable

Clin. Response - G - Good

Whenever several titers are given in one box, they usually represent results on successive serum specimens. Repeated testing of a serum is indicated by the letter R, preceding the titer. Neg. signifies 41:10.

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Dosage in tablets: C - Clinic; H - Home - Location of bubo. 100

Reg. - Regularity: R - Regular; I - Irregular; ? - Questionable Clinical Response: G - Good; F - Fair; P - Poor

successive serum specimens. Neg. signifies < 1:10. Whenever several titers are given in one box, they usually represent results on

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132 148	88	1	R	GB	20			50	10					
13 12	28	R	Ī	P					160		·			-5
21 24	1 28	7	I	5th day ruptured ^s	01									- -5

100 mg

Gland reappeared. Placed on 24 tablets Ilotycin (200 mg) and aureomycin 360; 60H with good response *Because of poor response, placed on chloromycetin 16C; 76H

f Enlarged left node, no pain. Given aurecmycin 90H

s - side effects

Dosage in capsules and tablets: C - Clinic; H - Home Loc. - Location of bubo Reg. - Regularity: R - Regular; I - Irregular Clinical Response - G - Good; P - Poor; F - Fair

Whenever several titers are given in one box, they usually represent results on successive serum specimens. Repeated testing of a serum is indicated by the letter R preceding the titer. Neg. signifies <1:10.

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CHLOROMYCETIN

SCORE		-	ļ.,	17-3	7		c	ķ		9		*			ماد		-	10		0	r.		77		
	19 - 30 Mo.				710										Neg.	0,0									
	13 - 18 Mo.			Neg.	OT.	3	C.	3)								1	-				8				
LTS	6½ Mo. 1 Tr.			10 Neg.	70*			Ş	250	3	Neg.	0.7		K	3	1	T			077	200	†	Neg.		
RESI	3≥ - 6 Mo.	10	97	Neg.	00 0	3			**01	25	707	017		7,7,70	#C%7#7#	160	10	08			10 (R Neg				•
Z.	1½ - 3 Mo.		2	070	320			0,1	0	Neg.	Nog	10	8			le le	97								4
LEMENT F	1 Mo.		077		320										•	0,7					108	1	1		
COMP	2 WK.		0,7	077	160			0,1	82		Neg.	017	OIL.	Ç.	}		6,							Neg • I	
	1 WK.	_																						$ brack \ $	
	Initial	10	20	8	160	10	077	077	20		10	077	07	0,1	}	07	10	9	160	80	&	Neg	- 20	neg.	
CLIN. RESPONSE		ტ	G.	हर ।	Ð	Unknown	G	ð	g	O	D.	ŋ	9	U	0	Ð	Ð	Unknown	Ð	ย	O.,	0	, e	5	
REG.		R	R	H	2 2		R	I	2	æ	2	œ	H.	F	R	R	R	I	R	6 4	H	+	-		
100.		R	R	Φ.	œ;	В	7	17	7	œ;	7	-3	P	1	1	В	Ţ	T.	7	L	н	1-1-1	+	1	ture
X -	•	5 40			52		32	-				170	1	_	07	_	-	_	-	-	_	1 22	122	711	1 stricture
NO.		10 16	-		15 32	73 24	-	75 28	-	<u>5</u>	+	87 8	7	г	12	_	_	-	-	-		36	1.		*Urethral
—	ŀ	<u>ا</u> ت	1	~ 1	I	<u></u>	<u> </u>		7		1	_	62	Ø	į	8	ο'	9	<u> </u>	킭	<u></u>	138	E	1	# #

**Right gland enlarged - Chloromycetin therapy repeated
***Reappearance of gland - Chloromycetin therapy repeated
Feland btill enlarged - placed on triplesulfonamide: 366; this
o Dysuria

Dosage in capsules: C - Clinic; H - Home
Loc. - Location of bubo
Reg. - Regularity: R - Regular; I - Irregular;
Response -Good;F-Falr; P-Poor ? - Questionede Clin.

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MAGNAMTCIN

SCORE		-5	₹.	9	~ ₂ -		r _o		-1		-5
	19 - 30 Mo.	Neg.	50	01							
	13 - 18 Mo.	Neg.	Neg.				Neg. (R 10)	01			
S	6½ Mo. 1 Tr.	10 Neg.	Neg.	90	Neg.					017	
st result	3½ - 6 Mo.		20 Neg.		Neg.			80			
COMPLEMENT FIXATION TEST RESULTS	1 Mo. 1½ - 3 Mo.	10	01	110	Neg.				320***	017	
ENT FIX	1 Mo.	*01	* O <u>†</u>		\$0\$				079		
COMPLEM	2 WK.	20		O [‡]	20						
	1 WK.										
	Initial	20	90	7.0	80	077	160		160		979
\$ CLIN.	RESTONDE	Q .	Q.	D	D.	Unknown	Œ	Ð	ſz ₄	უ	a ,
LOC. REG.		85	æ	<u>ر</u> .	æ	ć	æ	æ		æ	
100.		R	7	1	R	2	22	æ	17	17	В
DOSAGE	#	99	72	25	77	**2	122**	78**	30	% %	156**
DOS.	ပ်	36	20	21	877	**		**************************************	O Ž	817	
NO.		77	52	27	i R	IO.	103	106	OII	H	122

*Gland still enlarged. Placed on aureomycin. Good response

**100 mg. per capsule

***Enlargement of gland still present. Put on chloromycetin with good response. No follow-up available.

Dosage in capsules: C - Clinic; H - Home Loc. - Location of bubo

- Regularity: R - Regular; I - Irregular; ? - Questionable Reg. - Regularity: Clinical Response:

Whenever several titers are given in one testing of a serum is indicated by the letter R preceding the titer. Neg. signifies #1:10. box, they usually represent results on successive serum specimens. Repeated

SULFATHIAZOLE

								COMPLI	MENT FT	COMPLEMENT FIXATION RESULTS	RESULTS				
NO.	DOSAGE C. H.		.001	REG.	CLIN. RESPONSE	INITIAL	1 WK.	2 WK.	1 MO.	1 2 – 3 Mo.	3½ - 6 MO.	6½ мо. 1 тв.	13 - 18 MO.	19 - 30 MO.	SCORE
7		112	1	٤	Ð	20		10					10		0
Ħ	æ	701	H	ċ	įz,					017) JO	Neg Lo	10	10 (R Neg.)	4.0
23		78	-7	ċ	Q.	50				* 017	10 Neg.	Neg.			بر
O.T		78	R	2	Ъ					160%	07				-5
T.		30	R	ċ	Ь					**079	0179	091 079		07	<u>۲</u> -
57	017	108	В	R	Ð	07		017						Neg.	9
72		120	В	3	Ð			Neg.			10				77
85		36	R	8	Ð				Neg.	Neg.		Neg.			ង
87	24	56	В	格	9th day ruptured	0179				80					γ .⊐
776		90	e¥;	ક	Ъ				***OT	017	20 Neg.	Neg.			- -
308		72	¥	ં	P					14091	01			20	- 5
130		72	H	2.	_ ච					160	160	160	160		0
															,

*Gland still enlarged. Placed on triplesulfonamide.

**Gland still enlarged. Put on triplesulfonamide, sulfamethazine, and sulfathiazole - 20C; 84H. Poor response. After 20 days, put on aureomycin - 16C; 96H. Good response.

Good response. ***Gland still enlarged. Put on aurecmycin 366; 60H. Good response.

Dosage in tablets: C - Clinic; H - Home

Reg. - Regularity Clinical Response:

G - Good; F - Fair; P - Poor

Whenever several titers are given in one testing of a serum is indicated by the letter R, preceding the titer. Neg. signifies <1:10. box, they usually represent results on successive serum specimens. Repeated

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